PAIRED TUMOR AND GERMLINE MOLECULAR PROFILING:

GERMLINE FINDINGS AND THEIR CLINICAL IMPACT

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By
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CERTIFICATION OF APPROVAL

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ABSTRACT

Targeted next-generation DNA sequencing of paired tumor and germline DNA samples allows for detection of treatment-relevant variants in tumors with greater accuracy than tumor-only sequencing. In addition, this approach allows for the identification of cancer predisposition pathogenic variants in a patient's germline DNA. A records review was used to determine the rate of positive germline findings, and their clinical follow-up, in 600 consecutive pediatric and adult patients who underwent paired molecular profiling using the UCSF500 assay. The assay sequences the coding regions of ~500 targeted cancer-related genes. One hundred positive germline findings were flagged in 90 of 600 patients (15%). There were 38 types of positive findings, with sequence changes in *MUTYH* ($n = 15$), *CHEK2* ($n = 11$), *BRCA2* ($n = 10$), *BRCA1* ($n = 5$), and *TP53* ($n = 5$) occurring most frequently. In 67 (74%) of the cases, positive germline findings were previously unknown and genetic counseling was recommended. Of the 41 patients who have been seen by a genetic counselor at UCSF, 34 (83%) had their findings interpreted as autosomal dominant clinically actionable cancer risk variants. Patient family members in 10 of these cases had germline testing for the autosomal dominant findings; 8 individuals were found to have the familial risk variant and 13 had true negative results. The data reveals that paired tumor and germline DNA analysis uncovers actionable heritable traits in a significant fraction of cancer patients and represents the preferred approach to analyzing malignancies in children and adults.

INTRODUCTION

Tumor and germline genetic testing have been used separately in cancer treatment and prevention for the last two decades. Tumor genetic profiling is used to clarify a cancer diagnosis, guide treatment, and modify prognosis. Germline testing is used to identify individuals with inherited cancer risk variants and to inform their cancer prevention, screening and treatment recommendations. Recently, paired tumor and germline molecular profiling has become available for selected patients with cancer at several institutions. Paired testing identifies germline variants which facilitates more accurate interpretation of genetic changes seen in the tumor tissue and guides more appropriate treatment recommendations. The Clinical Cancer Genomics Laboratory at the University of California, San Francisco, has been offering paired testing since April of 2015. The germline findings identified by this test in the first two years of its clinical use are reported in this study. The clinical follow-up and impact of the identified germline findings are also described.

Somatic Testing

With the exception of hormone treatments, nearly all of the drugs used to combat cancer prior to the late 1990's functioned by killing cells that were replicating their DNA and dividing. While this is an effective strategy for combating fast-growing cancer, it also damages other tissues with rapid cell turnover like the blood-forming cells of the bone marrow, hair follicles, and the lining of the digestive tract.

The damage to other tissues is responsible for many of the side effects of traditional chemotherapy that patients find challenging and painful.

In the 1990's, a new chemotherapeutic strategy of targeted therapy began being used to combat specific malignancies. Building on previous research that elucidated cancer development by identifying oncogenes, tumor suppressors, and signaling pathways that were altered by genetic changes in tumors, researchers began looking for macromolecules that were integral to the malignant phenotype but not expressed in vital organs and other tissue. By co-opting these malignancy-specific molecular features, targeted therapy became safer and more effective than traditional chemotherapies for some cancers (DeVita & Chu, 2008).

The first successful targeted therapy was the treatment of chronic myelogenous leukemia (CML) with the BCR-ABL1 tyrosine kinase inhibitor, imatinib. Several types of leukemia are associated with a somatic translocation product known as the Philadelphia chromosome. The Philadelphia chromosome consists of portions of chromosome 22 and 9 and contains a fusion gene called *BCR-ABL1*. The fusion gene produces a tyrosine kinase protein that is constitutively on, which causes the cells to divide uncontrollably. Imatinib is an adenosine triphosphate-binding selective inhibitor of the *BCR-ABL1* protein product and its use has been shown to assist with complete and durable remission in the early chronic phase of CML with minimal toxic effect. Since it was approved by the FDA in 2001, imatinib's use has been expanded to the treatment of relapsed and metastatic gastrointestinal stromal tumors. Many other successful targeted therapies have come

to market since that time, for example, vemurafenib in BRAF-mutant melanoma and erlotinib in EGFR- mutated non-small cell lung cancer (Ross et al., 2004).

In order to effectively deploy targeted therapy, strategies for identifying somatic genetic changes in tumor tissue had to be developed. Initially, the somatic testing of a limited number of oncogenic markers involved the use of a number of technologies, including PCR, Sanger sequencing, mass spectrometric genotyping, fluorescence *in situ* hybridization and immunohistochemistry. However, due to the small amount of tissue obtained from biopsies and technical limitations, none of these techniques could be modified to address the increasing volume and variety of therapeutically relevant somatic alterations that occur across the genome.

Enter next-generation sequencing. In the last five years, several laboratories have used this technology to create multigene cancer profiling tests that examine hundreds of relevant genes. Somatic testing with massively parallel sequencing has allowed oncology to move further away from treating histopathologically defined disease with cytotoxic chemotherapy and towards a model in which a patient's tumor genetics informs the prescription of effective molecularly targeted drugs (Frampton et al., 2013). That being said, intratumor genetic heterogeneity continues to be a challenge in the application of targeted therapy. Within a tumor, there may be subclonal cell populations that do respond to a targeted therapy and those that do not. As such, after a period of clinical benefit, malignant progression may resume once the cells that don't respond to the targeted therapy become the majority (Gerlinger et al.,2012).

Germline Testing

During the same time period targeted therapies began being used, another major development was taking place in oncology. Researchers began identifying the underlying genetic germline causes of hereditary cancer syndromes. While some of these syndromes had been recognized since the 1960's, it was not until the 1990's that the culpable gene changes were identified. These discoveries allowed germline testing for inherited cancer predisposition to become a well-established part of care for individuals who may be at an elevated risk for cancers of the breast, ovary, colon, stomach, uterus, thyroid and other vital organs. The identification of a pathogenic germline variant in a patient often warrants a change in clinical management earlier and more intensive cancer screening or risk-reducing surgery. Germline testing for several cancer predisposition syndromes is now part of clinical guidelines and is often covered by insurance when clinical criteria are met (Robson, Storm, Weitzel, Wollins, & Offit, 2010).

While there are now over three hundred recognized inherited cancer syndromes, hereditary breast and ovarian cancer syndrome (HBOC) continues to be the most well-known and it will be discussed here to illustrate the benefits of germline testing. HBOC is most often caused by a germline pathogenic variant in the genes *BRCA1* and *BRCA2*. Unlike sporadic breast and ovarian cancer, which usually occurs post menopause, women with HBOC tend to be younger at the age of diagnosis. They are also at elevated risk for bilateral and multifocal disease. The general population's lifetime risk for breast and ovarian cancer is 13 and 1 percent

respectively. For women with a *BRCA1* pathogenic variant, the risk by age 70 is much higher, at 47-66% for breast cancer and 35-46% percent for ovarian cancer. Risk is similarly elevated for women with a *BRCA2* pathogenic variant, and men with a *BRCA2* pathogenic variant are at elevated risk for male breast cancer and prostate cancer (Ngeow & Eng, 2016).

When a woman is found to have a pathogenic *BRCA1* or *BRCA2* variant via germline testing, there can be changes to her clinical care whether or not she has a personal diagnosis of cancer. If a woman does not have cancer, she would be offered more intensive screening for breast cancer beginning in her twenties or thirties using MRI and mammography. Chemoprevention of breast cancer with tamoxifen and chemoprevention of ovarian cancer with oral contraceptives can also be considered. Risk-reducing mastectomy and salpingo-oophorectomy are likewise available for cancer prevention. If a woman has a breast or ovarian cancer diagnosis and is found to have *BRCA1* or *BRCA2* pathogenic variant, more aggressive surgery can be considered to reduce the risk for a second primary cancer. Poly-ADP ribose polymerase (PARP) inhibitors can also be used to treat cancers in which the action of the *BRCA1* or *BRCA2* protein product is compromised (Palma, Ristori, Ricevuto, Giannini, & Gulino, 2006).

The identification of a germline pathogenic variant can alter a patient's cancer screening, prevention, and treatment strategy. Additionally, their family members can have germline testing for the pathogenic variant. If a family member's test comes back negative, then they may be reassured that they are not at increased risk for

certain cancers. If a family member tests positive, however, then their cancer screening, prevention, and treatment can be modified to follow established high-risk guidelines.

Rational for Paired Testing

Somatic tumor and germline genetic testing are usually done separately. Recently, however, concerns have been raised about the ability of tumor-only profiling to accurately identify tumor-specific alterations because each tumor contains inherited (germline) and tumor specific (somatic) variants. As such, it is possible that a tumor-tissue-only test would falsely identify a germline change as a somatic change.

False positive somatic results are exactly what Jones et al. (2015) identified in their study. They analyzed tumor and germline samples from 58 individuals with several types of cancer. When they looked at only the tumor results, and used bioinformatics to remove sequencing/alignment errors and variants that are common in germline population databases, 23 and 33 percent of variants were incorrectly called as somatic using an 111 gene targeted panel and exome analysis respectively. Forty-eight percent of the patients had false positive somatic calls that could have been classified as actionable changes. For example, a *JAK2* variant was reported in the catalytic domain (Jones et al., 2015). Some somatic *JAK2* mutations contribute to the myeloproliferative process in lymphomas. Ruxolitinib is a targeted therapy that could be recommended to inhibit the altered *JAK2* protein product. However, there have been a few cases of patients with germline *JAK2* mutations that show resistance

to *JAK2* inhibitors like Ruxolitinib. As such, it would be clinically beneficial to clarify if the *JAK2* variant seen in the tumor was a somatic or germline mutation. If the variant was a germline change, it may impact efficacy of Ruxolitinib (Marty et al., 2014).

The UCSF500 Cancer Gene Panel

To avoid the false positive somatic results that tumor-only testing can produce, a number of institutions have begun using paired tumor and germline molecular profiling to identify which variants are specific to the tumor. Among these is the University of California, San Francisco (UCSF). The UCSF Clinical Cancer Genomics Laboratory (CCGL) offers an assay that is run on saliva or peripheral blood (germline) and tumor cells that are micro-dissected from formalin-fixed, paraffin–embedded tissue blocks. The assay is called UCSF500 and it is considered when: (1) diagnostic uncertainty remains after histological evaluation of the tumor, (2) the diagnosis does not have an efficacious standard- of-care therapies, and/or (3) The tumor or tumors progressed through prior therapies.

The assay targets the coding region of 510 cancer-related genes (Figure 1), the *TERT* promoter, select introns from 40 genes to detect fusion genes and other structural variants, and regularly spaced intergenic regions on each chromosome to allow for copy number assessment. Target enrichment is performed by hybrid capture using custom oligonucleotides. Sequencing is performed on an Illumina HiSeq 2500 and duplicate reads are removed so allele frequency and copy number can be determined accurately. The analysis is based on human reference sequence UCSC

build hg19 (NCBI build 37) and uses the following software packages: BWA, Samtools, GATK, CNVkit, Pindel, SATK, Annovar, Freebayes, Delly, and Nexus Copy Number. Single nucleotide variants and small insertions/deletions (indels) were verified by visualization with Integrated Genome Viewer. For samples at least 25% tumor, >200x coverage for the tumor sample, and >100x coverage for the normal sample, the sensitivity is 99% and 83% and the specificity is 98% and 71% for fully clonal single nucleotide variants and small indels respectively. Microdissection allows the sequencing sample to be enriched for neoplastic cells. Large rearrangements, deletions, and duplications detected by this assay are validated on an individual basis (Kline, Joseph, Gernert, van Ziffle, Talevich, et al., 2016).

Figure 1. Cancer-associated genes targeted for capture and sequencing on the UCSF500 Cancer Gene Panel

ABCB1	ABL1	ABL2	ACVR1	ACVR1B	ACVR2A	AJUBA	AKT1	AKT2	ATK3	ALDH7A1
ALK	APC	APOBEC3G	AR	ARAF	ARAFP1	ARHGAP35	ARID1A	ARID1B	ARID2	ARID5B
ASH2L	ASXL1	ASXL2	ATF1	ATM	ATR	ATRX	ATXN1	AURKA	AURKB	AXIN1
AXIN2	AXL	BAP1	BARD1	BCL2	BCL2A1	BCL2L12	BCL2L2	BCL6	BCL10	BCL11B
BCOR	BCORL1	BIRC3	BLM	BRAF	BRCA1	BTCA2	BRD4	BRIP1	BTK	B4GALT3
CALR	CARD11	CBFB	CBL	CCND1	CCND2	CCND3	CCNE1	CD163L1	CD79A	CD79B
CD274	CDC42	CDC73	CDH1	CDH2	CDH4	CDH5	CDH20	CDK12	CDK4	CDK6
CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHD4	CHEK1	CHEK2	CIC
CKS1B	CLDN18	COL1A1	COL2A1	CREBBP	CRKL	CSF1R	CSF3R	CTCF	CTNNA1	CTNNB1
CUL3	CUX1	CXCR4	CYLD	CYP2C8	CYP3A4	CYP3A5	DCC	DDIT3	DDR2	DDX3X
DICER1	DNMT3A	DOT1L	DPYD	DUSP2	DUSP4	DUSP6	DYNC1I1	EBF1	EDNRB	EGFR
EGR3	EIFA2	EIF1AX	ELF3	EMSY (C11orf30)	EP300	EPCAM	EPHA3	EPHA5	EPHA6	EPHA7
EPHB1	EPHB4	EPHB6	EPPK1	ERBB2	ERBB3	ERBB4	ERCC2	ERCC6L	ERG	ESPL1
ESR1	ESR2	ETS1	ETV6	EWSR1	EZH1	EZH2	FAM123B (WTX)	FAM46C	FAM58A	FANCA
FANCC	FANCE	FANCF	FANCG	FANCL	FBXO43	FBXW7	FGF10	FGF14	FGF19	FGF23
FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4	FH	FHIT	FLCN	FLT1
FLT3	FLT4	FOXA1	FOXA2	FOXL2	FOXO1	FOXP1	FOXP4	FSIP1	FUBP1	FUS
FYN	GAB2	GAK	GATA1	GATA2	GATA3	GID4 (C17orf39)	GIGYF2	GIPC3	GLI1	GIL2
GNA11	GNA13	GNAQ	GNAS	GPR124 (ADGRA2)	GPSM1	GRIN2A	GRM3	GSK3A	GSK3B	GSTP1
GUY1A2	H3F3A	H3F3B	H3F3C	HDAC9	HGF	HIF1A	HIST1H1C	HIST1H3B	HNF1A	HOXA3
HOXB13	HRAS	HSPA2	HSPA5	HSP90AA1	ICK	ID3	IDH1	IDH2	IGF1R	IGF2R
IKBKE	IKZF1	IKZF2	IKZF3	IL36A	IL7R	INHBA	IRF4	IRS2	JAK1	JAK2
JAK3	JAZF1	JUN	KAT6A (MYST3)	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KIT	KLF4
KLHL6	KMT2A	KMT2B	KMT2D	KRAS	LARS	LEF1	LIFR	LRP1B	LRP6	LRRK2
LTK	MAFB	MALAT1	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAP3K2	MAP3K5	MAP3K7	MAP3K9
MAPK1	MCL1	MCTP1	MDM2	MDM4	MECOM	MED12	MEF2B	MEN1	MET	MGA
MIR142	MITF	MLH1	MPL	MRAS	MRE11A	MSH2	MSH6	MTOR	MUTYH	MYB
MYBL1	MYC	MYCL	MYCN	MYD88	MYH9	NAV3	NBN	NCOR1	NF1	NF2
NFE2L3	NFE2L2	NFKBIA	NFKBIE	NIPBL	NKX2-1	NOTCH1	NOTCH3	NPM1	NRAS	NSD1
NTSC2	NTRK1	NTRK2	NTRK3	NUP93	NUP98	NUTM1	ORSL1	PAK3	PALB2	PAX3
PAX5	PAX8	PBRM1	PCBP1	PDCD1LG2	PDGFB	PDGFRA	PDGFRB	PDK1	PDS5B	PHF6
PHLPP2	PIK3CA	PIK3CG	PIK3R1	PIK3R2	PIM1	PKHD1	PLCG1	PLCH2	POLD1	POLE
POLQ	POT1	POU3F2	PPM1D	PPP2R1A	PPP6C	PRDM1	PRKACA	PRKAR1A	PRKCA	PRKDC
PRX	PTCH1	PTCH2	PTEN	PTK2B	PTPN1	PTPN11	PTPRD	PTRK	PTPRT	PYDC2
RAC1	RAC2	RAD21	RAD50	RAD51	RAD51C	RAD51D	RAF1	RARA	RASA1	RB1
RBM10	REL	RELA	RET	RHEB	RHOA	RHOT1	RICTOR	RIT1	RNF43	ROS1
RPL5	RPTOR	RRAS	RRAS2	RUNX1	RUNX1T1	SDHB	SDHD	SETBP1	SETD2	SF3B1
SGK1	SH2B3	SHH	SHOC2	SIN3A	SLITRK6	SMAD2	SMAD3	SMAD4	SMARCA2	SMARCA4
SMARCB1	SMC1A	SMC3	SMG7	SMO	SNX31	SOCS1	SOS1	SOS2	SOX9	SOX10
SOX17	SOX2	SPEN	SPOP	SPRED1	SPRY1	SPRY2	SPRY4	SPTA1	SRC	SRSF2
SS18	STAG1	STAG2	STAT3	STAT4	STAT6	STK11	SUFU	TACC1	TADA1	TADA2B
TAS2R60	TBL1XR1	TBX3	TBX22	TCF3	TCF4	TCF7L2	TCL1A	TERT	TET2	TFE3
TFEB	TGFBR2	TLR4	TNC	TNFAIP3	TNFRSF14	TNKS	TNKS2	TOP1	TMPRSS2	TP53
TP63	TPMT	TRAF7	TRPM1	TRPM3	TRRAP	TSC1	TSC2	TSHR	TSHZ2	TSHZ3
U2AF1	UGT1A1	UGT1A7	USP7	USP9X	VAT1L	VEZF1	VHL	WAPAL	WHSC1	WISP3
WRN	WT1	XPO1	XRCC2	XRCC3	XRCC5	XRCC6	YAP1	YWHAE	ZFHX3	ZMYM3
ZNF217	ZNF668	ZNF703	ZRSR2							

Figure 1. Genes highlighted in red indicate those for which select intronic regions are also captured, in addition to all coding exons, for detection of gene fusions and other structural variants.

The UCSF500 results are written up in formal reports by molecular pathologists. The reports describe the somatic findings and any pathogenic and likely pathogenic germline findings. Germline variants are reported if classified as pathogenic or likely pathogenic in ClinVar and confirmed by a CCGL molecular pathologist. For variants not in ClinVar, truncating variants in well-established tumor suppressor genes are reported if present in less than 1% of the publically available 1000 Genome or Exome Sequencing Project datasets. If the pathologist identifies a germline variant that is not clearly pathogenic or likely pathogenic but seems consistent with the clinical picture, they may report it so that it is reviewed further by the provider. The reports also state whether the germline findings are associated with any known tumor predisposition, whether the paired results have diagnostic and prognostic implications, and whether there are appropriate targeted therapies for the tumor. Results are discussed at a weekly molecular tumor board meeting that is attended by oncologists, surgical and molecular pathologists, genetic counselors, and surgeons.

A team at Memorial Sloan Kettering hospital recently published their germline findings for a similar test offered at their institution. Of the 1566 patients tested, 13% had presumed pathogenic germline variants in genes associated with cancer susceptibility in their germline sample. The patient's cancer type was concordant with the germline altered cancer susceptibility gene in only 41% of the cases. The Memorial Sloan Kettering study was conducted anonymously (Schrader et al., 2016). This study on the UCSF500 was not conducted anonymously. As such, the

clinical follow-up for the germline results and family member testing outcomes will also be described.

METHODS

This research was conducted via a retrospective review of records available at one large medical center in Northern California, the University of California, San Francisco. A list of CCGL cases with their respective medical record numbers and positive germline findings is updated each week. Cases included in this study had reports issued between April 1st, 2015, and March 31st, 2017. The case list was reviewed to conduct the study. Cases in which the positive germline finding was exclusively an alteration in *TMPT*, which indicates thiopurine drug response, or an incidental finding unrelated to cancer were removed. Removal was justified because these findings were not reported for the entirety of the first two years the UCSF500 was being used. Non-cancer related incidental findings stopped being reported because the consent form did not include these types of findings as possible results.

For the remaining cases, UCSF500 result reports, UCSF electronic medical records and UCSF Cancer Genetics and Prevention Program internal databases (Tracker and Progeny) entries were reviewed. The following additional data were extracted for each patient: CCGL report number, cancer diagnosis, personal and family cancer history on record prior to UCSF500 test, variant classification, related tumor result, treatment recommendation based on germline finding, report mention of genetic counseling, sex, age at time of test, date UCSF report was issued, date of genetic counseling referral, date of genetic counseling, counseling recommendations, number of family members seen for genetic counseling, number of family members

who had germline testing, and family member germline test results. This data was used to answer the following research questions:

1.   What were the positive germline findings (i.e., those findings classified as pathogenic and likely pathogenic, or flagged for further review)?

2.   Was there evidence of germline finding involvement in the tumor, or was the finding identified incidentally?

3.   In cases with germline tumor involvement, did the patient have a cancer type for which their germline finding is known to increase risk?

4.   Were their new treatment strategies based on the germline findings?

5.   Did the patient meet clinical (NCCN and published consensus) criteria for germline genetic testing before using the UCSF500?

6.   Were patients who had positive germline results referred to genetic counseling by their test-ordering providers?

7.   Were patients seen by a genetic counselor?

8.   Were screening and prevention recommendations updated, based on the germline findings, in the genetic counseling appointment?

9.   Were patient family members seen by a genetic counselor and did they have germline testing?

In the analysis stage, altered genes were considered surprising in the tumor type to which they contributed if the altered gene did not have an established increased risk for that tumor type upon literature review. National Comprehensive Cancer Network (High-Risk Breast and Ovarian, and Colorectal) and additional published germline testing criteria (e.g. Neurofibromatosis 1 and Fanconi anemia testing criteria) were consulted during the review of the patient's personal and family

cancer histories to establish if criteria for germline testing were met prior to the UCSF500. Criteria met and germline variants were considered concordant if the gene the variant was in is commonly included in multigene panels used to assess the tumor type or the suspected hereditary cancer syndrome. Inclusion was assessed by reviewing panel tests listed on genetests.org.

To explore possible reasons that some patients with positive germline findings were not referred to genetic counseling, two Fisher exact tests were employed to see if the results report's mention of genetic counseling and select variant types impacted referral rate respectively. All tests of significance were two-sided, with a $p < .05$ considered as significant. These tests were performed using IBM's SPSS 24 statistical analysis software.

This research project has UCSF IRB approval under study number 16-21070 and CSU, Stanislaus, IRB approval under protocol number 1617-108.

RESULTS

The UCSF500 Cancer Gene Panel was used in 629 clinical cases between April of 2015 and April of 2017. In 19 (3%) cases only tumor tissue was submitted. In another 10 (2%) cases the return of germline findings was declined by the patient. In these cases, germline changes were not highlighted in a separate report section, but the variants stayed in the report if they appeared in the tumor. For the remaining 600 cases the assay was run on, tumor tissue and peripheral blood or saliva samples were submitted and somatic and positive germline results were reported.

Positive Germline Findings

One hundred germline variants potentially related to cancer predisposition were reported in 90 (15%) cases. Of these positive findings, 82 (82%) were called pathogenic, 7 (7%) were called likely pathogenic, and 11 (11%) were flagged for further review. Adult patients made up 72% (n=65) of these 90 cases and pediatric patients made up the remaining 28% (n=25). The average age of the adult patients was 54.60 years (SD=14.13) and the average age of the pediatric patients was 8.08 years (SD=5.20). The patient tumor types fell into 22 categories with central nervous system tumors accounting for 36% (n=32) of the total number of cases with germline findings. The next most represented tumor types were colorectal, melanoma, and prostate with 10, 5, and 5 cases respectively (Fig. 2)

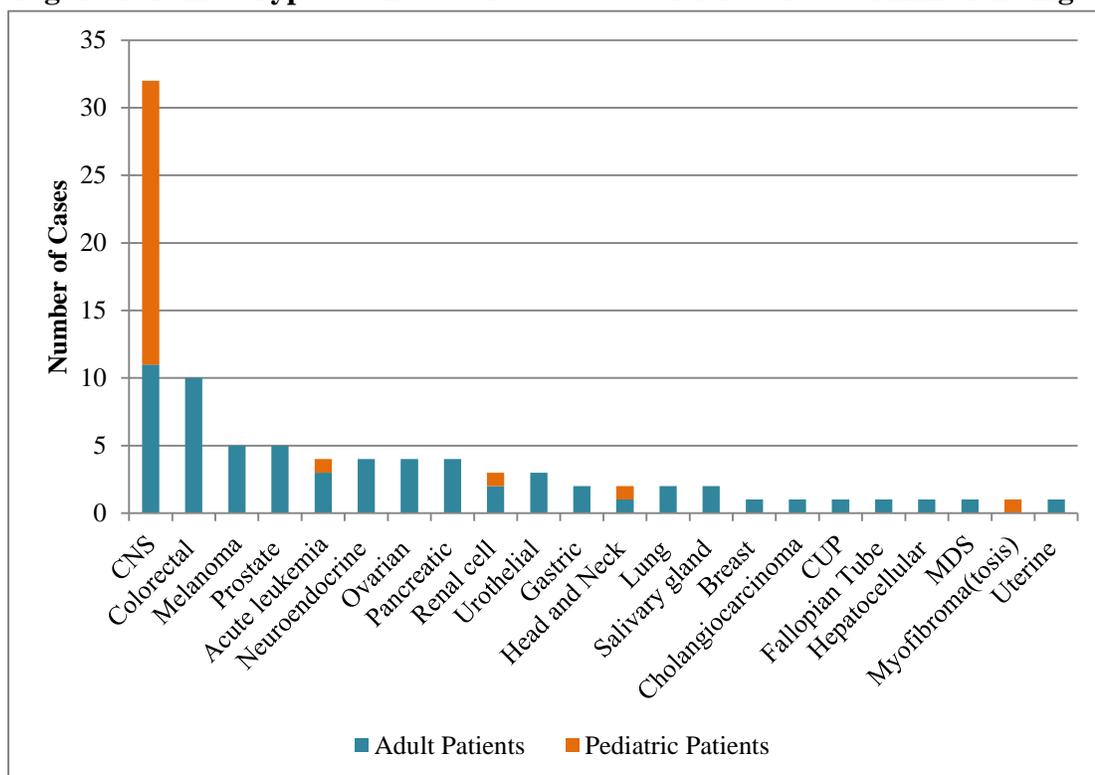**Figure 2. Tumor Types of the UCSF500 Cases with Positive Germline Findings**

Figure 2. CNS= Central Nervous System, CUP= Carcinoma of Unknown Primary, MDS= Myelodysplastic Syndrome

There were 38 types of positive germline findings identified, of which, 35 were sequence variants in individual genes (Figure 3). Those that were not single gene sequence variants included two instances of Trisomy 21, a deletion of *CDKN2B* and *CDKN2A*, and amplification of *RAF1*(4 to 5 copies suggested by assay). The instances of Trisomy 21, the most frequent cause of Down syndrome, were previously known, but their identification demonstrates the assay's ability to recognize copy number variation. The cancer predisposition genes altered most frequently were *MUTYH*, *CHEK2*, *BRCA2*, *BRCA1*, *TP53*, and *APC*, accounting for 16% (*n*=16), 12% (*n*=12), 10%(*n*=10), 6% (*n*=6), 5%(*n*=5), and 4%(*n*=4) of the total identified

sequence variants respectively. The only variant identified in *APC* was p.I1307K.

This polymorphism does increase colorectal cancer risk and occurs in 10% of the

Ashkenazi Jewish population (Boursi et al., 2013). However, p.I1307K does not

cause familial adenomatous polyposis syndrome like other pathogenic variants in

APC.

Figure 3. Frequency of Each Type of Positive Germline Finding Identified
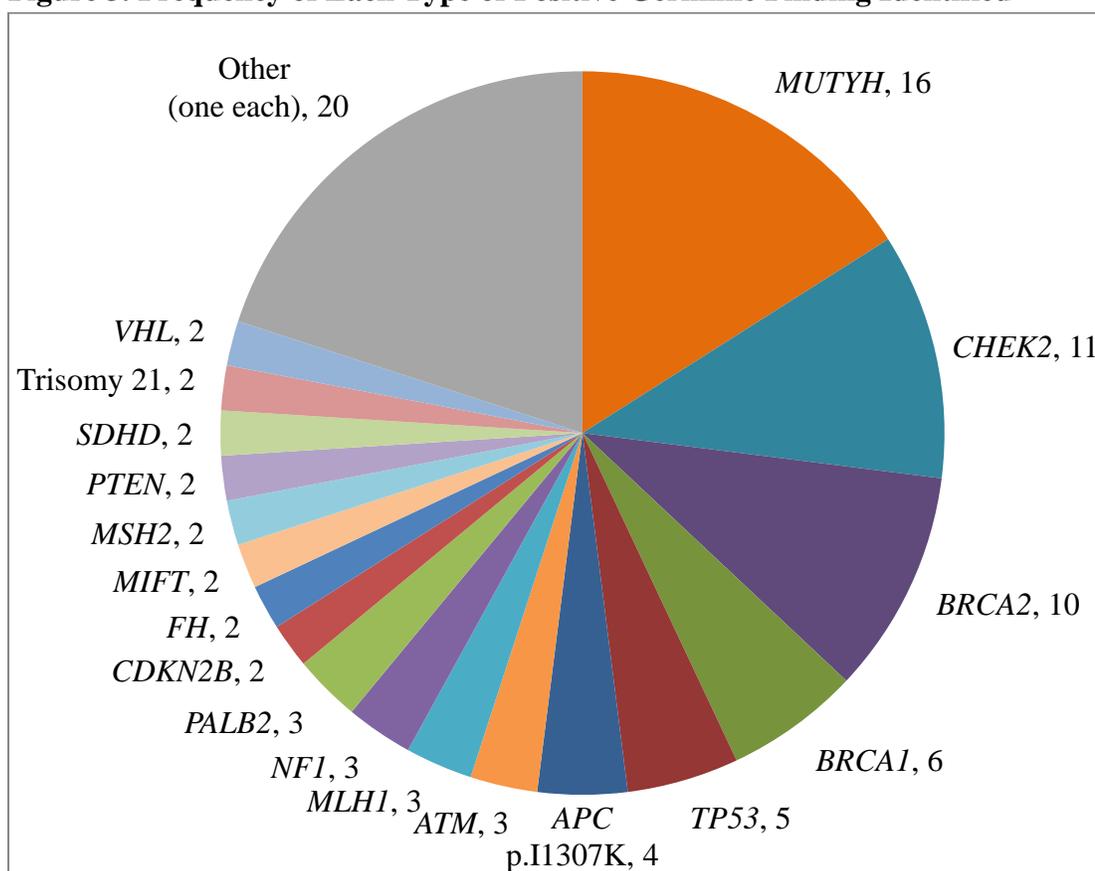

Figure 3. Sections labeled with gene names represent the number of cases with
sequence changes identified in that gene. The variants that occurred once each
include *RAF1* amplification, *CDKN2A* and *CDKN2B* deletion, and sequence changes
in *BLM*, *BRIP*, *ERCC2*, *FANCC*, *FANCG*, *HNF1A*, *HOXB13*, *MSH6*, *PDGFRB*,
PTCH1, *PTCH2*, *RAD50*, *SUFU*, *TERT*, *TGFBR2*, *TSC2*, *WISP3*, and *WRN*

In 52% (*n*=47) of the cases, the germline findings appear to be incidental, as there was no evidence they were involved in tumorigenesis. In the other 43 cases (48%) the germline findings appeared to have contributed to the cancer, i.e., biallelic germline mutations, trisomy 21, loss of heterozygosity in the tumor tissue, and a somatic change in the second allele. In 17% (*n*=8) of the cases that showed germline finding involvement in tumors, the observed tumor type was discordant with the tumors that are expected with the altered cancer risk gene (Table 1). This suggests that the cancer risks related to these genes may not be fully understood and that more studies are needed to clarify if the observed cancers occur more frequently in patients with pathogenic variants in these genes.

Table 1

Tumors with surprising altered gene positive findings

Tumor	Altered Gene	Tumors Expected with Pathogenic Changes in the Altered Gene
Glioma	*ATM* (P, Copy-neutral LOH)	Breast and Pancreatic
Colonic adenocarcinoma	*BRCA1* (P, Copy-neutral LOH)	Breast and Ovarian
Glioblastoma	*CHECK2* (P, Hemizygous)	Breast and Colorectal
Pancreatic Neuroendocrine	Monoallelic *MUTYH* (P, Copy-neutral LOH)	Colorectal
Pancreatic Neuroendocrine	Monoallelic *MUTYH* (P, LOH unspecified)	
Astrocytoma	Monoallelic *MUTYH* (P, Hemizygous LOH)	
Prostate	*PALB2* (P, LOH unspecified)	Breast and Pancreatic
Medulloblastoma	*PALB2* (P, Hemizygous LOH)	

Note. Several germline variants showed loss of heterozygosity (LOH) in tumor types for which they are not known to increase risk. Copy-neutral LOH means that there has been no net change in the allele copy number. Hemizygous LOH is caused by a deletion of the other copy of the gene.

Based on identified positive germline findings alone, 21% (*n*=19) of patients had recommended changes in therapy. Somatic results informed additional changes, but those are outside the scope of this study. Recommendations indicated by the germline findings included avoiding the use of radiation and use of PARP inhibitors in two cases each, and the use of unique targeted therapy in 15 cases.

Criteria Assessment

Pathogenic or likely pathogenic germline findings were known prior to using the UCSF500 due to previous genetic testing in 15 patients. Eight patients had very limited records at UCSF because their care occurred elsewhere. The remaining 67

patients (74% of the total 90) had newly identified positive germline findings and had treatment records at UCSF. Of these, 58% (*n*=39) did not meet the established criteria for germline testing based on the personal health histories and family histories recorded in their charts prior to testing with the UCSF500.

The distribution of those germline changes found in the cases meeting clinical criteria and those not meeting clinical criteria can be seen in Figure 4. Not surprisingly, variants in high-risk cancer genes like *PTEN*, *MLH1*, *BRCA1*, and *BRCA2* account for more of the variants in the group meeting criteria than the group not meeting criteria. Likewise, changes that confer low to moderate cancer risk, like single risk variants in *MUTYH*, *CHEK2*, *PALB2*, and *ATM*, were more often altered in the group not meeting established germline testing criteria. However, one pediatric and one adult patient with *TP53* variants, categorized as pathogenic and likely pathogenic respectively, were in the group of patients who didn't meet established criteria. Given the penetrance of *TP53* pathogenic changes and the high risk for cancer that they confer, this is surprising. However, this could be explained by de novo changes in these individuals; 7-20% of TP53 pathogenic germline mutations are de novo (Gonzalez et al., 2009).

Figure 4. Positive Germline Findings in Patients Meeting and Not Meeting Germline Testing Criteria
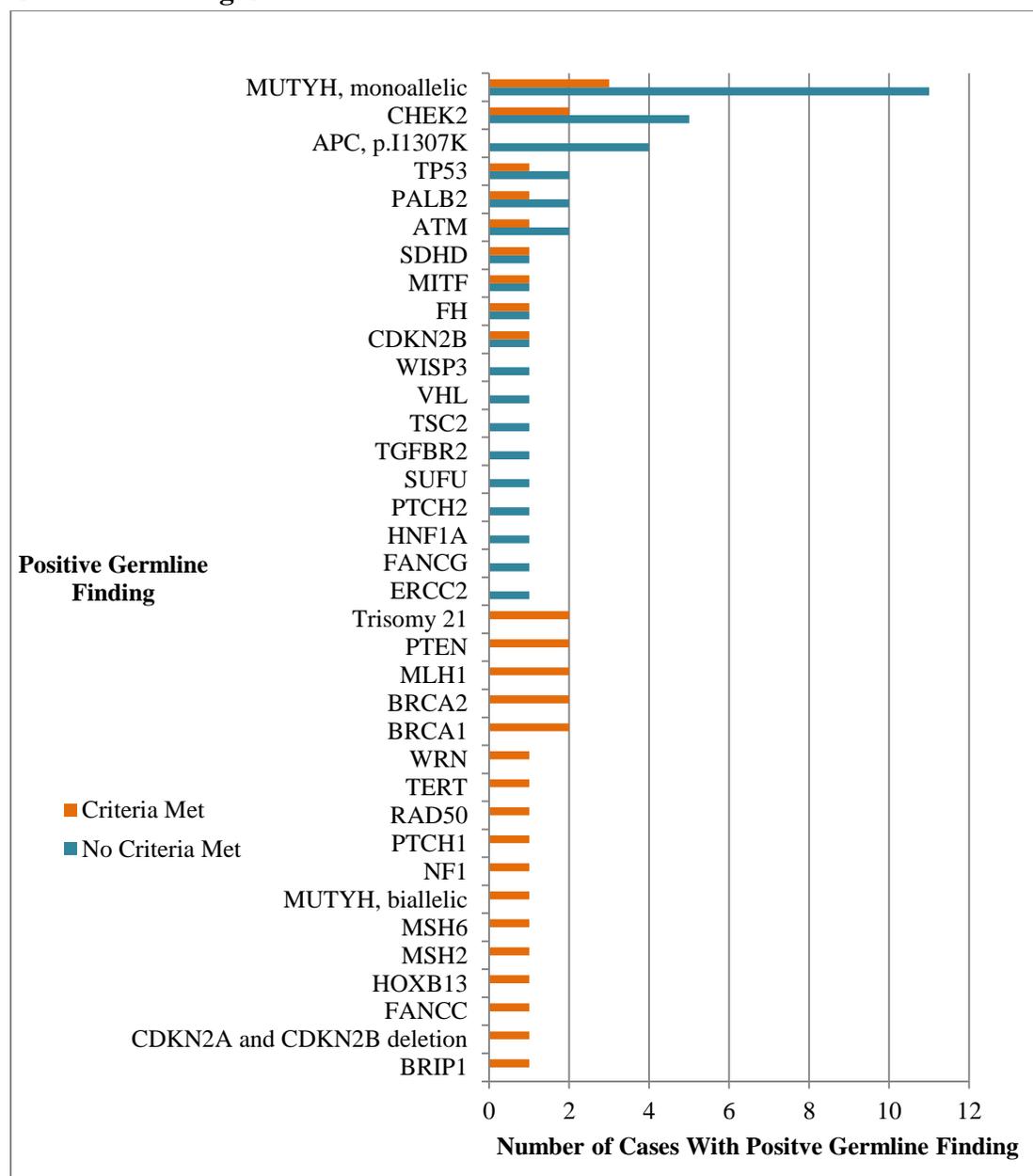
Of the patients who did meet criteria, panel germline testing based on the patient's diagnosis and family history may not have included all the genes that were ultimately identified with variants in 7 cases (Table 2). In these cases, some of the

germline findings were surprising given the clinical picture. For instance, a *BRCA1* pathogenic variant was identified in a man who had synchronous colorectal cancers and a grandmother with pancreatic cancer. Given his personal and family history, identifying a genetic risk factor for gastrointestinal cancers might be expected. Instead, he was found to carry a pathogenic variant that greatly increases the risk for breast and ovarian cancer.

Table 2

Cases with Unexpected Positive Findings Given the Germline Testing Criteria Met

Patient Cancer	Relevant Family History in Chart	Criteria Met	Genes with variants	Cancer associated with unexpected altered gene
Prostate (Gleason 7)	Father: Prostate Mother: Breast, Skin Sister: Breast, Melanoma	NCCN, *BRCA*	*MITF* (P)	Melanoma and Renal
Endometrial, previous ovarian	Mother: Breast	NCCN, *BRCA*	*MUTYH* (LP)	Colorectal
Gastric, previous breast	Father: Prostate Mother: Breast Sister: Breast Mat. Aunt: Breast	NCCN, *BRCA*	*MUTYH* (P)	Colorectal
Synchronous colorectal	Pat. Grandmother: Pancreatic	NCCN, Lynch	*BRCA1*(P)	Breast and Ovarian
Colon	Father: Colon, Prostate Pat. Aunt: Colon Pat. Grandmother: Colon	NCCN, Lynch	*MSH2* (P) (Expected) **And** *HOXB13* **(P)**	Prostate
Ovarian, known *BRCA2* pathogenic variant	Sister: Breast Aunt: Breast	NCCN, *BRCA*	*BRCA2* (P) (Known) **and** *FH* **(LP)**	Kidney
Glioma, clinical dx. of NF1	none	NIH, NF1	*CHEK2* (P)	Breast and Colorectal

Note. P= Pathogenic, LP=Likely Pathogenic, Mat.= Maternal, Pat.= Paternal, dx.= diagnosis, NF1= Neurofibromatosis 1

Clinical Follow-Up

Referral to genetic counseling when a positive germline finding was identified relied on the discretion of the ordering provider. Of the 67 patients with treatment records at UCSF, 78% (*n*=52) were referred on to genetic counseling for assessment or follow-up. In several cases, a referral to genetic counseling was made prior to the

release of the UCSF500 report or care was previously established because the clinical

and/or family history looked suspicious for a hereditary cancer syndrome. In 22%

(n=15) of cases, no referral was made.

Of those 52 patients who were referred or had care established 77% (n=41)

have been seen by a genetic counselor. Of the remaining 11 cases, 5 patients declined

counseling, 4 patients have appointments scheduled in the future, 1 patient's

insurance would not cover the appointment, and 1 patient had established care with

genetics prior to UCSF500 testing, but has not been seen to discuss his UCSF500

germline finding.

For the 41 patients who were referred for the first time to genetic counseling

after their UCSF500 report was issued, the referral took place between 0 and 302

days after the report was released (M=33.12, SD=53.93). Of the 34 of these who have

gone on to be seen by a genetic counselor, the appointment took place between 0 and

183 days after the referral was placed (M=43.53, SD=45.91).

Forty-one patients have been seen in genetic counseling to review their

UCSF500 germline results. Of these patients, 83% (n=34) have had their germline

finding interpreted as autosomal dominant pathogenic, or likely pathogenic, cancer

risk variants in genetics clinic. Five patients (12%) had their finding interpreted as a

variant of uncertain significance (VUS). Four of these variants were initially flagged

for further review in the UCSF 500 report and one variant, called likely pathogenic,

was interpreted as a VUS in genetics clinic. Two (5%) patients were identified as

carriers of autosomal recessive conditions -- Fanconi Anemia and Chuvash

Polycythemia. Chuvash Polycythemia does not confer additional risk for cancer, but its carrier state is identified by the UCSF500 because it is caused by a variant in the *VHL* gene. Other pathogenic changes in the *VHL* gene cause the hereditary cancer condition Von Hippel-Lindau syndrome.

Of the 34 patients with a newly identified autosomal dominant cancer risk variant, 23 were adult patients and 11 were pediatric patients. The frequency of the various autosomal dominant cancer risk variants in the adult and pediatric patients can be seen in figure 5. Of the adult patients, 52% (*n*=12) had new screening recommendations made for them based on their germline results. Five others were directed to follow their oncologist's recommendations, as they were currently in treatment for, or had previously had, the cancer for which they were at elevated risk. One patient, who had a monoallelic *MUTYH* likely-pathogenic variant, was advised to have further germline testing because the clinical picture and family history were suggestive of Lynch syndrome and *PMS2* is not reported on by the UCSF500 test.

Figure 5. Autosomal Dominant Cancer Risk Variants in Adult and Pediatric Patients Who Were Seen for Genetic Counseling
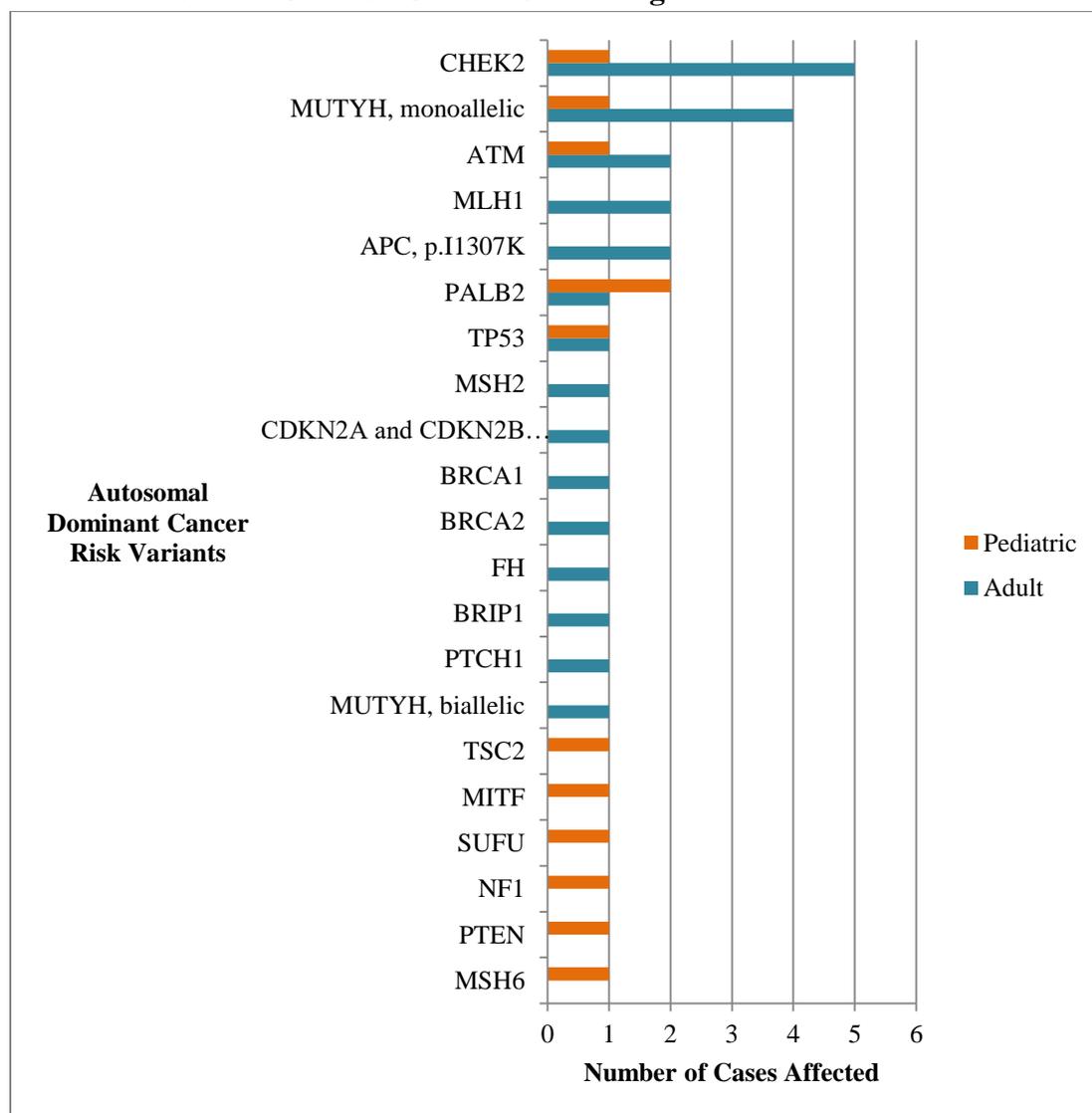
Referral of family members to genetic counseling was recommended to all of the adults with a confirmed autosomal dominant risk factor. Family members of 5 (22%) of these adult patients were seen by a genetic counselor according to notes in medical records. In three cases, the family members declined to have their own

germline genetic testing at the time of the appointment. In one case the UCSF500 report was requested from an outside institution so that germline testing could be arranged for a patient's sister. In the last adult family case, the two adult children of a man with Lynch syndrome had germline testing and were not found to share their father's pathogenic variant.

Among the 11 pediatric patients with an autosomal dominant cancer risk variant, 46% (n=5) had adjustments made to their childhood screening recommendations and 46% (n=5) were advised to reconnect with genetics in adulthood for up-to-date adult screening recommendations. In one case that was seen at Kaiser in the spring of 2015, the child's family was advised that a monoallelic pathogenic *MUTYH* variant did not warrant additional screening and follow-up in the absence of a family history of colorectal cancer. In each of these 11 pediatric cases, some family members were seen for genetic counseling. In 8 of these cases (73%) family members had germline testing for the identified variant. Six parents and two extended family members were identified as sharing the pathogenic or likely pathogenic variants identified in the patients. Their screening and prevention recommendations were updated accordingly. Eight parents and three siblings had testing that showed they did not share the patient's clinically relevant variant. In the previously discussed case at Kaiser, family testing was not recommended. Testing was not indicated for one family because other members did not have clinical features of Neurofibromatosis 1, and one other family declined genetic testing.

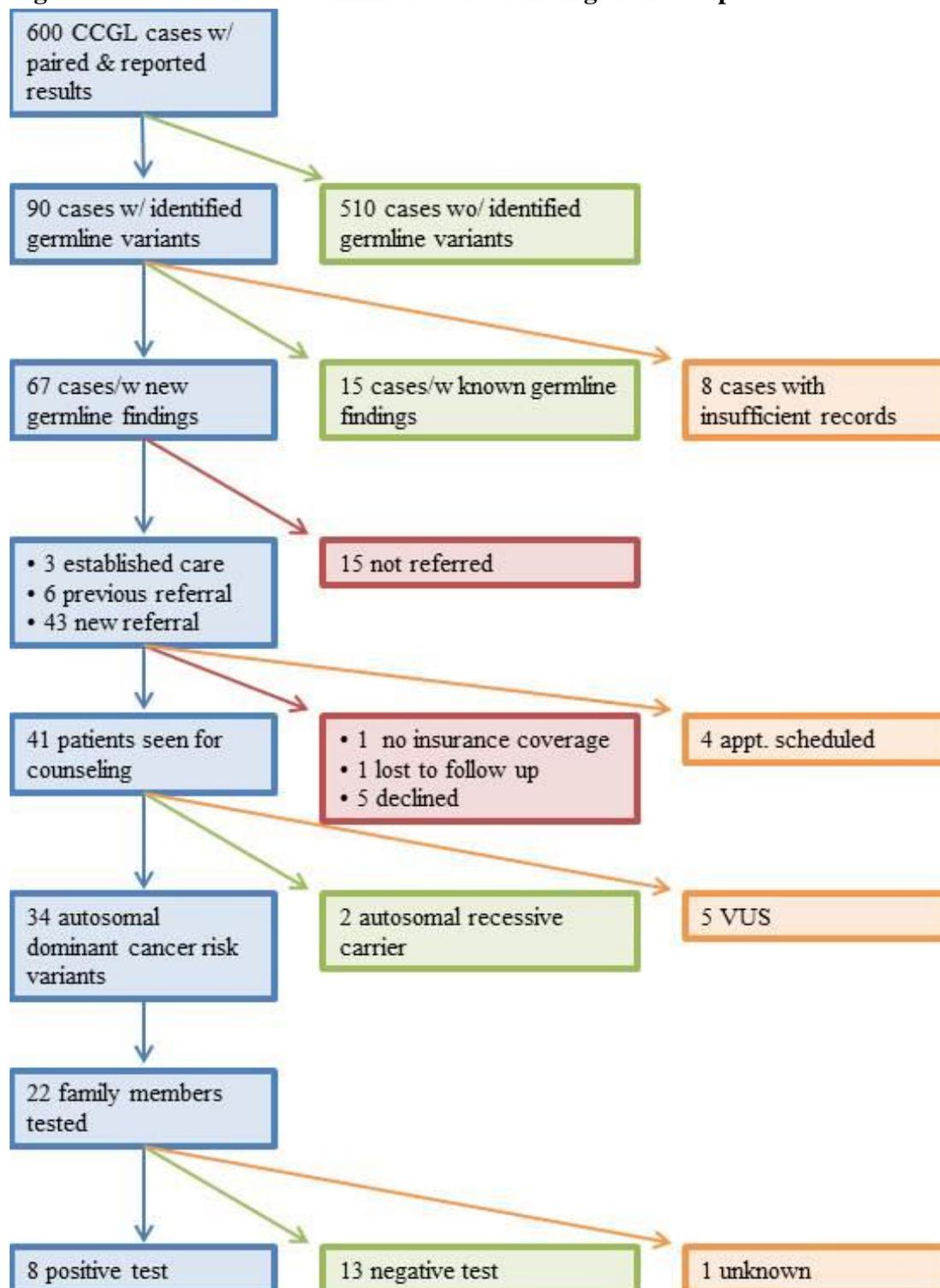**Figure 6. Flow Chart of Germline Positive Findings Follow-up**

Figure 6. VUS= variant of uncertain significance

No Referral

As mentioned before, in 15 cases, there was no referral to genetics for counseling about the germline findings. Chart notes provided insight into the reason for this in some cases. In one case, the patient had died before the test results were reported. In two cases the patient was moved to hospice shortly after the report was released. The majority of one patient's care was taking place in Chicago. In two cases, the doctors offered to make a referral and are waiting for the patient to indicate whether or not they are interested in being referred. In one case, the doctor indicated an intention to refer on three occasions, but the referral was never made and the patient has since died.

To explore some reasons a referral to genetic counseling may not have been made, result reports were reviewed to see if genetic counseling was mentioned in all cases with a germline result. Counseling was not mentioned in 28% of the reports of patients with confirmed new findings. In order to see if this had an impact on referral a Fisher's exact test was run (Table 3). No significant association was identified (p=1.00).

Table 3

Contingency table showing how many patients were referred to genetic counseling after their report was issued with and without mention of genetic counseling

Was Genetic Counseling Mentioned in the Patient's UCSF500 Results Report?				
		yes	no	total
Was a Referral to Genetic Counseling Made?	yes	31	12	43
	no	11	4	15
	total	42	16	58

Observationally, it appeared that individual with monoallelic *MUTYH* variants or the *APC*, p.I1307K polymorphism accounted for a large proportion of those not referred. To see if these genetic changes were less likely to illicit a referral, another Fisher's exact test was run (Table 4). Again, no significant association was identified ($p=.059$). However, this test was closer to being significant and may become significant in the future as the number of cases grows.

Table 4

Contingency table showing how many patients were referred to genetic counseling when their germline variant was a monoallelic change in MUTYH / APC p.I1307K or not

Was the Patient's Variant a Monoallelic Change in *MUTYH* or *APC*, p.I1307K?				
		yes	no	total
Was a referral to Genetic Counseling Made?	yes	6	37	43
	no	6	9	15
	total	12	46	58

DISCUSSION

The purpose of this study was to report the positive germline findings that were identified by the UCSF500 paired tumor and germline molecular profiling assay. The germline findings' impact on patient care and patient family members was reviewed as well. Fifteen percent of patients in this study had positive germline findings identified. A similar test, offered to patients with advanced disease, at Memorial Sloan Kettering identified cancer risk variants in13% of patients (Schrader et al., 2016). It is estimated that 5-10% of cancer occurs in individuals who have inherited a pathogenic, cancer-predisposing variant (Garber & Offit, 2005). The percentages identified in this study and by Schrader et al. (2016) exceeded that. In both paired testing studies, however, the samples were enriched for patients with advanced disease; this may explain the discrepancy. Individuals with hereditary cancer predispositions tend to have cancer at younger ages than is typical. Less cancer screening occurs with young people and young people with symptoms may not present to care in a timely fashion because they think cancer is unlikely. Both of these factors could contribute to cancer being identified at more advanced stages in individuals with hereditary cancer syndromes. For example, early-onset colorectal cancer is more likely to present at advanced stages and to be caused by Lynch syndrome or MUTYH Associated Polyposis syndrome (Giraldez et al., 2010).

The present study suggests that the germline finding contributed to tumorigenesis in 48% of the cases. Schrader et al. (2016) found this number to be

31

21% in their study. The discrepancy between the percentages reported in these studies may be a result of the study design. In the Schrader et al. (2016) study, the ordering provider received the tumor results and the germline findings were only collected by the researchers. The UCSF500 assay provides somatic and germline results to the ordering provider. As such, this UCSF500 test population may be enriched for patients whose providers were suspicious of germline contributions to tumors prior to testing. In investigating contribution to tumorigenesis, both studies report on loss of heterozygosity and second somatic pathogenic hits in the functional allele. It is possible that there are other molecular mechanisms, such as epigenetic silencing of the functioning allele, that were not evaluated and could have allowed additional monoallelic pathogenic variants identified in these studies to contribute to tumorigenesis.

The seven tumors reported in Table 1 showed tumor involvement of germline changes that are not currently thought to contribute to elevated risk for the types of cancer in which they were found:

- This series identified a patient with glioma who had a germline pathogenic variant in *ATM* with copy-neutral loss of heterozygosity in her tumor. *ATM* pathogenic variants are known to increase risk for breast cancer and pancreatic cancer, and one study suggests elevated risk for colorectal and stomach cancer, but no studies link them to increased risk for glioma (Roberts et al., 2012; Thompson et al., 2015).

- A germline *BRCA1*pathogenic variant showed copy-neutral LOH in the tumor

  sample of a 53-year-old man with synchronous primary colorectal cancers.

  BRCA1 pathogenic variants increase risk for breast and ovarian cancer and

  one recent study suggests they increase risk for early onset colorectal cancer

  in women under 50 (Mersch et al., 2015; Sopik, Phelan, Cybulski, & Narod,

  2015). Elevated risk for colorectal cancer risk in men with BRCA1

  pathogenic variants has not been reported.

- The UCSF500 identified a germline *CHEK2* pathogenic variant, which was

  hemizygous in the tumor sample, in a patient with Glioblastoma. *CHEK2*

  pathogenic variants are known to confer elevated risk for breast cancer and

  colorectal cancer, and some studies suggest elevated risk for kidney, thyroid

  and prostate cancer (Cybulski et al., 2004; Xiang, Geng, Ge, & Li, 2011).

  Bell et al. (1999) describe a family with a clinical diagnosis of Li-Fraumeni

  Syndrome caused by 1100delC *CHEK2* pathogenic variant. Two members of

  this family had gliomas, but elevated risk for CNS tumors has not been

  confirmed by other studies. However, Simon et al. (2006) show that *CHEK2*

  polymorphisms do correlate with glioblastoma prognosis.

- Two patients with pancreatic neuroendocrine tumors and a patient with

  astrocytoma had germline, monoallelic, pathogenic variants in *MUTYH* that

  showed LOH in their tumors. Monoallelic *MUTYH* pathogenic variants are

  known to increase risk for colorectal cancer and the Win et al. (2016) large

  study demonstrates added extracolonic risk for gastric, hepatobiliary,

endometrial, and breast cancer. Win et al. (2016) did not identify added risk

for cancer of the brain, pancreas, kidney or prostate. However, a recent study

on 102 pancreatic neuroendocrine tumors did find a larger than expected

proportion of pathogenic *MUTYH* germline variants. Also, there has been

one pediatric case report with pathogenic *MUTYH* germline variants in a

child with astrocytoma, in addition to the published case of the pediatric

patient in this study (Aghajan, Levy, Newbury, and Crawford, 2016; Kline,

Joseph, Grenert, van Ziffle, Yeh, et al., 2016; Scarpa et al., 2017).

- Germline pathogenic *PALB2* variants showed LOH in a patient with prostate

  cancer and a patient with medulloblastoma. Single pathogenic variants in

  PALB2 are known to increase risk for breast and pancreatic cancer (Jones et

  al., 2009 and Antoniou et al., 2014). One specific *PALB2* pathogenic variant

  has been seen in a patient with familial prostate cancer, but others, including

  the variant identified by the UCSF500, have not been shown to increase risk

  (Pakkanen et al., 2009). Biallelic *PALB2* pathogenic variants, causing

  Fanconi anemia subtype n, have been shown to increase risk for

  medulloblastoma, but monoallelic pathogenic variants have not (Reid et al.,

  2007).

 These seven tumors, and several of the aforementioned studies, suggest that more

studies are needed to elucidate the full range of cancer risk caused by pathogenic

changes in cancer risk genes. Paired tests like the UCSF500 could be used to

contribute to such research as more patients with germline variants are identified.

Larger numbers of patients need to be identified for such studies to produce statistically significant results.

While tumor-specific changes are usually sought to guide treatment, germline variants contributed to treatment recommendations in 21% of cases in this study. This reveals an additional advantage to using a paired testing strategy. While using PARP inhibitors used to be the only, non-hormonal, targeted treatment common among patients with certain germline changes, more recently palbociclib has been used to treat tumors with *CDKN2A* alterations (Elvin et al., 2017; Goa, Adams, & Swain, 2015), which can be germline and cause melanoma-astrocytoma syndrome, and sirolimus has been employed as an anti-tumor agent in patients with *PTEN* germline changes, i.e., Cowden syndrome (Cloughesy et al., 2008; Schmid et al., 2014). Two recent reviews have also come to the conclusion that identifying germline changes is becoming more useful in directing treatment; specifically for Lynch syndrome, Gorlin syndrome, Tuberous Sclerosis complex, and Multiple Endocrine Neoplasia type 2 (Agrwal et al., 2014; Iyevleva & Imyanitov, 2016).

Fifty-eight percent of patients with confirmed, newly identified germline findings did not meet established criteria for germline genetic testing based on the information in their medical records. Using this assay identified pathogenic germline alterations in patients who may have never otherwise had germline testing. That patients with pathogenic germline variants are not meeting criteria suggests that testing criteria may be too stringent. As the cost of testing continues to go down, it may become economically justifiable to relax criteria so that more individuals with

hereditary risk factors for cancer can be identified. It is also possible that the reported cancer risk and penetrance for some of these hereditary cancer genes will be modified as lower a-priori risk individuals are included in larger studies.

Among those patients who met established germline testing criteria, some had pathogenic germline findings identified that may not have been found if they had panel testing informed by their cancer diagnosis and family history alone. Cases in which risk factors unrelated to the present clinical picture, and that increase risk for other malignancies, are identified could be used to argue for the application of cross-cancer panels for all patients seeking to test. While some argue that this would be population screening, others argue that cancer is a manifestation of a genetic disease, and therefore, most patients with cancer should have germline genetic testing to screen for a hereditary disposition. Additionally, targeted panel testing and cross cancer panel testing are a similar price at this point in time, so comprehensive cancer risk assessment makes economic sense. However, cross cancer panel testing may lead to the identification of pathogenic variants in high-risk cancer genes that come with standard recommendations for aggressive risk-reducing surgery in patients who have no known family history or clinical features. In such cases, risk assessment and making informed screening recommendations can be challenging.

A substantial portion of the patients who went to genetic counseling after having positive germline findings on the UCSF500 test had changes made to their cancer screening recommendations and/or had family members tested for their pathogenic or likely pathogenic variant. This exemplifies the clinical utility of this

test as short-term screening recommendations were modified for 52% of adult patients and 42% of pediatric patients. Family testing is also an excellent outcome of including the germline portion of this assay. It was interesting to note, however, that only 8% (n=2) of the adult patients have records of family germline testing at UCSF, whereas 73% (n=8) of the pediatric patients had family who tested. This may not be surprising given that at least one family member of pediatric patients has to attend their appointments. As such, some family communication about hereditary cancer risk will take place. Following up with adult patients about family communication can generally present more challenges because adults are more often geographically removed from their blood relatives, or may be estranged. Family communication for adults having testing with the UCSF500 may be particularly challenging given that these patients all have a current cancer diagnosis. Family communication about hereditary cancer risk may not rank among their priorities during treatment or hospice care.

Twenty-two percent of patients with newly identified positive germline findings were not referred to genetic counseling and one patient who had previously established care with a genetic counselor has not had any follow up for his UCSF500 results. Without seeing a genetic counselor, these patients may not be able to reap the benefits of the germline dimension of this test described in the previous paragraph. Relying on clinician initiative for referrals to be made does not appear to result in consistent referrals. An automatic referral protocol could increase the downstream clinical benefits of the UCSF500 test for patients and their family members. Ordering

providers may have rationales for why they did not refer certain patients to genetic counseling. Identifying these reasons could facilitate constructive conversations between genetic counselors and ordering providers.

IMPLICATIONS FOR PRACTICE

This retrospective records review project showed that a paired tumor and germline assay can identify positive germline findings in a significant number of patients seeking treatment for unusual and/or advanced cancers. These findings can guide treatment and screening recommendations for patients and lead to family member germline testing when referrals to genetic counseling are made. This study also elucidated areas for improvement in the reporting and clinical flow at UCSF. However, the experience at UCSF and the recommendations for improvement could inform the practice of any institution planning to use a paired tumor and germline test. Ideal implementation involves collaboration between the laboratory, oncologists, and clinical genetics.

The clinical utility of the CCGL UCSF500 reports could be improved through further standardization. Currently, referral to genetic counseling is not included in all reports with positive germline findings. Referral may not be necessary in cases where the germline findings were previously known, but a recommendation for genetic counseling referral should be standard for those patients with new findings. Loss of heterozygosity (LOH) for the positive findings is reported, but whether the LOH is copy-neutral or is the result of hemizygosity is not included consistently. LOH by either mechanism can contribute to tumorigenesis. However, the contribution is more tenuous in cases of variant hemizygosity in tumors with many different copy number

gains and losses. Specifying the type of LOH can clarify the likelihood that the positive finding is actually driving the tumor.

The clinical impact of this test could be improved at UCSF if an automatic referral protocol were to be instated. This could increase the number of referrals for patients with new positive findings and make sure that they happen in a timelier manner. As reported above, the referrals to genetic counseling took place between 0 and 302 days after the report was released, with an average intervening period of approximately a month (M=33.0, SD=53.9). Given how ill some of these patients are, any added wait time for a referral may prevent them from being seen for genetic counseling before they pass away. Patients do have the option of allowing a specified individual access to their results in the case of their death on the UCSF500 consent form, but family communication may be easier to facilitate when a patient is still alive.

This records review provided insight into family counseling and follow-up testing that occurred at UCSF, and in a few cases, other nearby institutions. Information about family follow-up elsewhere is not currently tracked. To ensure that family communication is taking place, it would be helpful if there was a way to track this information, especially for the family members of adult cancer patients. Kintalk, a family communication website developed by UCSF, is commonly recommended during genetic counseling at UCSF as a tool for sharing information with family members. If patients could consent for Kintalk to automatically update the cancer genetics database when a patient shares their result and/or pedigree with a family

member, family communication could be recorded. Patients who haven't shared their results could be given reminders and additional support to encourage family member education.

Tumor and germline paired assays like the UCSF500 are primarily sought to inform the treatment of the current cancer diagnosis. However, they also identify pathogenic germline variants. As genetic counselors are the clinicians who have the expertise in germline findings, and often have the privilege of explaining them to patients, they need to have a seat at the table when tests like this are deployed. As this study reveals, genetic counseling is crucial for getting the largest clinical impact out of the positive germline findings, as these appointments are where screening and prevention recommendations are changed and family communication is encouraged. Genetic counselors can help design the protocols that will get the appropriate patients to their office. This starts by making sure reports recommend genetic counseling and automating the referral process. Genetic counselors can further increase the positive clinical impact of this test by following up to see if family communication has occurred, so appropriate family members can have germline testing.

LIMITATIONS OF THE STUDY

This study has several limitations. The first of which relates the technical specifications of the UCSF500 assay. It is possible that the 600 patients who had paired tumor and germline testing have additional pathogenic germline variants that were not identified by the UCSF500. UCSF500 is not validated to identify intermediate-sized (single exon level) deletions and duplications. Also, several hereditary cancer syndrome causative genes, notably *PMS2,* have pseudogenes that can make confirming variants in them technically difficult (Vaugh et al., 2010). The CCGL has not implemented the additional methodology to address these technical challenges and so does not report findings in these genes. As such, additional pathogenic germline variants can be missed by this test.

In this study, establishing if patients met criteria for germline testing prior to using the UCSF500 relied partially on family cancer histories as they were recorded in electronic medical records (EMRs) prior to the date of the test. It is possible that these family histories could have been incomplete. Often, it is not until a patient meets with a genetic counselor that their family cancer history is recorded in a systematic and thorough manner. The possibility that more patients met criteria for germline genetic testing prior to using the UCSF500 than were captured by this analysis cannot be ruled out. Family histories not reported in the EMRs may have brought down the number of cases meeting germline testing criteria.

This study reported on the clinical impact of the positive germline findings discovered via the UCSF500 test. Family follow-up that took place at UCSF and nearby institutions was reported as part of this clinical impact. However, patient family follow-up that may have taken place at other medical centers would not be captured by the records that were reviewed. More patient family members may have had genetic counseling and germline testing as a result of the UCSF500 germline findings than were ascertained through the methods used in this study.

CONCLUSIONS

Paired germline and somatic molecular profiling, via the UCSF500 assay, identified positive germline findings in 15% of patients during the first two years of the test's clinical use. There was evidence to suggest the positive germline findings contributed to tumorigenesis in approximately half of these cases. Identified germline variants influenced treatment recommendations for several patients.

Many of the patients who had positive germline findings did not meet established criteria for germline testing prior to testing with the UCSF500. Paired germline and somatic molecular profiling identified patient with positive germline finding who may not have come to clinical genetics attention otherwise. Cancer screening and prevention recommendations were changed for ~50% of patients who were seen by a genetic counselor. Follow-up family member germline testing identified several family members who shared pathogenic and likely pathogenic changes with patients, and their screening and prevention recommendations were adjusted accordingly.

Paired germline and somatic molecular profiling identifies germline variants that influence treatment decisions, alter cancer screening and prevention recommendations, and help identify family members who are at elevated risk for cancer due to a shared pathogenic variant. As such, paired tumor and germline testing represents the preferred approach to analyzing genetic features of malignancies in

children and adults. The clinical impact of germline findings is greatest when

individuals with new findings are referred to genetic counseling.

FUTURE STUDIES

While paired tumor and germline molecular profiling is superior to tumor-only testing, it is also more expensive. Cost-benefit analysis is needed to see if the health care savings and downstream revenue from additional cancer screening and prevention are sufficient to justify the cost of this paired testing modality. Other institutions, that do tumor-only testing, are attempting to establish guidelines that instruct clinicians on which tumor results warrant germline testing follow-up (Ngeow & Eng, 2016). If the guidelines that are produced allow for the identification of a large fraction of patients with pathogenic germline changes, then this may become the more economically sustainable approach to germline testing for cancer patients.

In order to reap the maximal clinical benefit from a paired tumor and germline test, patients with positive germline findings need to be referred to genetic counseling. No referral occurred in 22% of the cases with new, positive germline finding in this study. A survey of ordering providers should be conducted to learn why these referrals were not made. Also exploring provider experience with delivering germline results, when their objective in ordering the test was to generate treatment recommendations, could help elucidate challenges with using this test in clinical practice.

In addition to the provider experience changing as a result of using paired testing instead of tumor-only testing, the patient's experience can be significantly altered. A study comparing the experiences of patients who learn about pathogenic germline

cancer risk variants through paired testing and those who learn about them through a germline test after a cancer risk pretest counseling session could teach genetic counselor about patient challenges specific to the paired testing process. With paired testing, identifying pathogenic germline variants is not the primary objective, directing treatment is. Patients may choose paired testing because they want the best information when making cancer treatment decisions, but they may not consider the full ramifications of potentially finding a pathogenic germline change before they take the test. As such, learning about a pathogenic variant may be more challenging for someone in a paired testing scenario than for someone who sought out hereditary cancer risk information through genetic counseling.

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